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Introduction

Phenoxy acid herbicides related to plant growth hormone are groundwater contaminants originating not only from agricultural practice, but also from landfill leachate.

Old landfills are major sources of multiple groundwater contaminants entering surface water by groundwater discharge.

We previously determined that the mineralization potential of phenoxy acids at field-relevant concentration depends on the herbicide mass discharges and initial abundance of *tfdA* gene classes (Batıoğlu-Pazarbaşı *et al.*, 2012).

In our current study, the effect of landfill leachate-contaminated groundwater on bacterial diversity of groundwater-surface water transition zones was studied at much higher concentration (120 mg kg^{-1}) and compared with field-relevant concentration scenario.

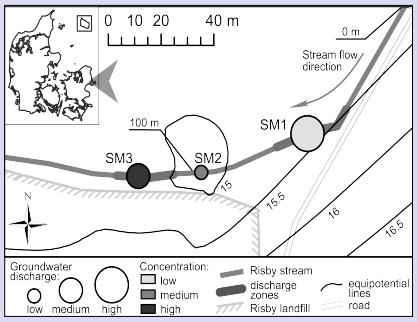
Hypothesis

The understanding of landfill impact on streambed sediments due to groundwater discharge is highly important to secure stream water quality. At our sampling site, the streambed sediment has been receiving herbicide mixtures at variable discharge rates for years.

We therefore hypothesize that this long-term *in situ* phenoxy acid herbicide exposure influences the bacterial community composition and *tfdA* gene diversity.

Methods

Map showing the sampling locations of the Risby Landfill and the groundwater discharge zones



SM: Seepage Meter, 1: 1st discharge zone, 2: 2nd discharge zone and 3: 3rd discharge zone

Streambed sediments were sampled from around the seepage meters installed in three discharge zones of the Risby stream impacted differently by leachate from the nearby Risby landfill.

We previously determined that SM3-streambed sediment received the highest mass discharge of phenoxy acid herbicide residues followed by SM2 and SM1 (Batıoğlu-Pazarbaşı *et al.*, 2012).

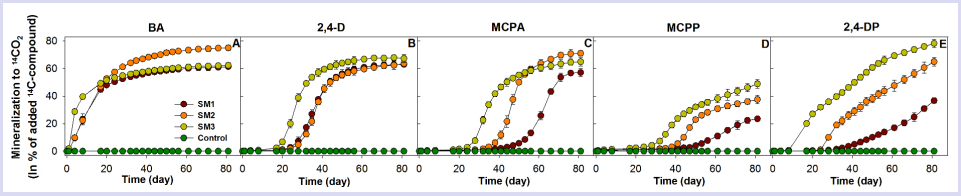
The landfill leachate entering the sediments contains phenoxy acid herbicides besides other typical landfill leachate compounds (Batıoğlu-Pazarbaşı *et al.*, 2012).

The sediment samples were transferred to microcosms and used for quantification of mineralization potential.

The sediment samples were collected before, during and after mineralization of field-relevant and higher concentrations and stored at -20°C for molecular analysis (multiplex qPCR, T-RFLP and pyrosequencing.

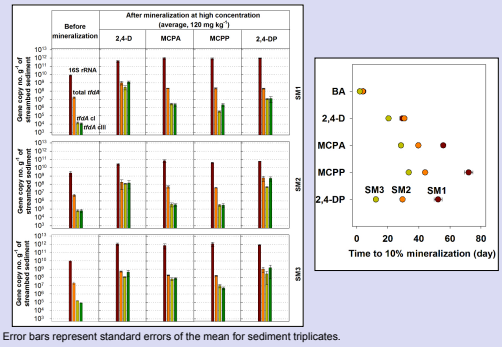
Results

Mineralization curves of (A) benzoic acid, (B) 2,4-D, (C) MCPA, (D) MCPP and (E) 2,4-DP in the streambed sediments of three discharge zones at 120 mg kg^{-1} concentration



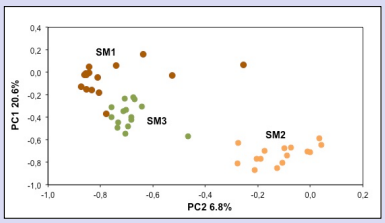
Error bars represent standard errors of the mean for sediment triplicates, some error bars are smaller than the symbol size.

The initial abundance of 16S rRNA, total tfdA, tfdA class I and class III genes before mineralization and the changes in gene abundance in the stationary phases of mineralization at 120 mg kg^{-1} concentration



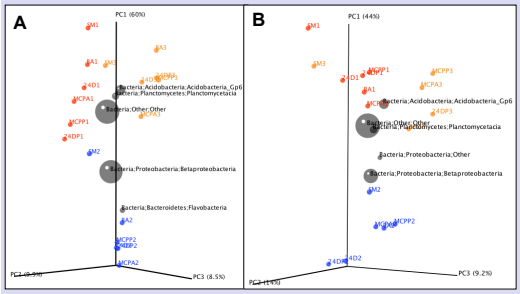
Error bars represent standard errors of the mean for sediment triplicates.

Principal Component Analysis (PCA) on relative abundance of T-RF peaks for the streambed sediments before mineralization and after mineralization at 8.5 µg kg^{-1} (control) and 120 mg kg^{-1} phenoxy acid concentrations



The average values of sediment triplicates were used in PCA analysis.

3D-biplots of principal coordinates visualizing quantitative phylogenetic measure of beta diversity (weighted UniFrac) based on pyrosequencing data before mineralization and after mineralization at (A) 8.5 µg kg^{-1} as control experiment and (B) 120 mg kg^{-1} phenoxy acid concentrations with the most abundant class-level taxa identified



Conclusions

The most rapid mineralization of phenoxy acid herbicides in SM3 and slower mineralizations in SM2 and SM1 were observed depending on the landfill impact on discharge zones.

The highest increase in *tfdA* gene classes as bacterial response was mostly observed in SM3 receiving the highest herbicide-contaminated groundwater discharge. However, in SM2 and SM1, higher abundance of *tfdA* gene classes for each discharge zone was observed where the shorter time needed to achieve 10% mineralization of 2,4-D and 2,4-DP than those of MCPA and MCPP indicating the higher affinity of degraders to 2,4-D and 2,4-DP.

Principal component analyses of both relative abundance of T-RF patterns and quantitative beta diversity of bacterial communities based on the pyrosequencing data disclosed similar clustering according to discharge zone.

Our study demonstrates that specific bacterial communities proliferate in streambed sediments in response to changing discharge rates of landfill-phenoxy acids.

Our results also indicate that bacterial responses of streambed sediment in each discharge zone differentiated after mineralization at field-relevant and higher concentrations despite of their similar clustering depending on the discharge zone.

Specifically, members of *Betaproteobacteria* (*Comamonadaceae*) increased in pyrotag read abundance with mineralisation in sediments from the first discharge zone, while reads affiliated to *Acidobacteria Gp6* were enriched in sediments from the third discharge zone after mineralization at field-relevant concentration. Under high herbicide concentrations, the relative abundance of *Alphaproteobacteria* (*Sphingomonadaceae*) enriched after incubation in sediments from the first discharge zone.

In the second discharge zone, pyrotags related to *Betaproteobacteria* (*Comamonadaceae*) and certain *Flavobacteria* increased in abundance at low herbicide concentration, while *Flavobacteria* were enriched, to a lesser extent, at high concentration.

These comprehensive findings based on integrated genomic-metagenomic approach are highly important to provide microbial ecological indicators to perform more accurate and advanced human health and ecological risk assessments and to protect stream water quality.

Acknowledgements

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Reference

Batıoğlu-Pazarbaşı M, Milosevic N, Malaguerra F, Binning PJ, Albrechtsen, H-J, Bjerg PL & Aamand J (2012) Discharge of landfill leachate to streambed sediments impacts the mineralization potential of phenoxy acid herbicides depending on the initial *tfdA* gene classes (Submitted)